PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 31/565, 31/575, A61P 25/28

A1

(11) International Publication Number: **WO 00/41700**

(43) International Publication Date: 20 July 2000 (20.07.00)

(21) International Application Number: PCT/DK00/00020

(22) International Filing Date: 17 January 2000 (17.01.00)

(30) Priority Data:
PA 1999 00050 18 January 1999 (18.01.99) DK

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: USE OF ESTROGENS AND DELTA–GONADIEN–21–OL–3,20–DIONES IN THE TREATMENT OR PROPHYLAXIS OF CEREBRAL DEGENERATIVE DISORDERS

(57) Abstract

The present invention relates to the use of a combination of estrogens or SERMs with delta–gonadien–21–ol–3,20–diones for the treatment of patients suffering from cerebral degenerative disorders, e.g. Alzheimer's disease, and prophylaxis hereof.

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USE OF ESTROGENS AND DELTA-GONADIEN-21-OL-3,20-DIONES IN THE TREATMENT OR PROPHYLAXIS OF CEREBRAL DEGENERATIVE DISORDERS

FIELD OF THIS INVENTION

The present invention relates to the use of a combination of estrogens or SERMs with delta-gonadien-21-ol-3,20-diones for the treatment of patients suffering from cerebral degenerative disorders, e.g. Alzheimer's disease, and prophylaxis hereof. The present invention also embraces pharmaceutical compositions and kits comprising these compounds and methods of using the compounds and their pharmaceutical compositions.

BACKGROUND OF THIS INVENTION

In classifying diseases of the nervous system, it is customary to designate a group of them as degenerative, indicating that they are characterized by gradually evolving, relentlessly progressive neuronal death occurring for reasons that are still largely unknown. The identifications of these diseases depends upon exclusion of such possible causative factors as infections, metabolic derangements, and intoxications. A considerable proportion of the disorders classed as degenerative are genetic. Others, however, occur only sporadically as isolated instances in a given family. Classification of the degenerative disorders cannot be based upon any exact knowledge of etiology or pathogenesis; their subdivision into individual syndromes rests on descriptive criteria based largely upon neuropathologic and clinical aspects. Many of the degenerative nervous system diseases progress uninfluenced by therapeutic measures.

Alzheimer's disease (AD) is perhaps the most important of all the degenerative diseases because of its frequent occurrence and devastating nature. AD is a degenerative brain disorder characterized clinically by progressive loss of memory, cognition, reasoning, judgement and emotional stability that gradually leads to profound mental deterioration and ultimatively death. AD is the most common cause of progressive mental failure (dementia) in the elderly and is believed to represent the fourth most common medical cause of death in the United States. The disease is currently estimated to affect about two to three million individuals in the United States alone. To date, many of the degenerative nervous system diseases, including AD, progress uninfluenced by any therapeutic measures.

The outstanding pathologic feature is death and disappearance of nerve cells in the cerebral cortex. This leads ultimatively to extensive convolutional atrophy, especially in the frontal, parietal, and medial temporal regions. Two kinds of microscopic lesions are distinctive for the disease. The first, originally described by Alzheimer, consists of intraneuronal accumulations of filamentous material in the form of loops, coils, or tangled masses referred to as Alzheimer neurofibrillar tangles. Their exact nature is currently under active investigations, but the neuropathologic evidence strongly suggests that these fibrillar masses of amyloidogenic nature are of major importance in bringing about the death of the neurons. The second histopathologic change that characterizes AD is the presence of intracortical clusters of thickened neuronal processes, both axons and dendrites.

Several lines of evidence indicate that progressive cerebral deposition of particular amyloidogenic proteins, -amyloid proteins (AP), plays a pivotal role in the pathogenesis of AD and can precede cognitive symptoms by years or decades (Selkoe DJ, Neuron 6:487, 1991). Recently, it has been shown that AP is released from neuronal cells grown in cell culture and is present in cerebrospinal fluid (CSF) of both normal individuals and AD patients (Seubert et al., Nature 359:325, 1992).

Biochemical studies show that choline acetyltransferase, the key enzyme required for the synthesis of acetylcholine, is decreased in the cerebral cortex in AD. The major source of the neocortical cholinergic innervation is a group of neurons situated in the basal part of the forebrain just beneath the corpus striatum, the nucleus basalis of Meynert. This nucleus is a site of major neuronal loss and of frequent Alzheimer neurofibrillar tangles. Thus, impairment of cholinergic transmission may play a part in the clinical expression of the disease. However, attempted therapy with cholinomimetic agents have been largely unsuccessful. In contrast, recent studies have indicated that estrogen influences cholinergic function through a stimulation of choline acetyltransferase (Kaufman H et al., Brain Res 453:389, 1988) and that it also increases the binding sites of hypothalamic nicotinic acetylcholine receptors (Morley BJ et al., Brain Res 278:262, 1983). Furthermore, it has been suggested that low dose estrogen replacement therapy may have a beneficial effect on AD (Okura T et al., Menopause 1:125, 1994).

There remains a need in the art for combined compositions and methods that are useful in the treatment or prophylaxis of degenerative cerebral disorders including Alzheimer's

disease. There is a further need for such compositions that lack or has decreased undesirable side effects of estrogen(s) and/or progesterone(s).

One object of the present invention is to provide compositions in one dosage form which can effectively be used in the treatment or prophylaxis of cerebral degenerative disorders, e.g. Alzheimer's disease.

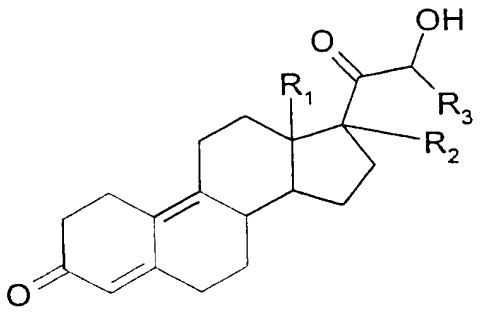
Another object of the present invention is to provide compositions, method of treatment or kits exhibiting a synergistic effect.

A further object of the present invention is to provide compositions, method of treatment or kits exhibiting no substantial side effects, such as high level of coronary heart disease events.

Other objects of the present invention will become apparent upon reading the present description.

DESCRIPTION OF THIS INVENTION

The present invention is based in part on the discovery that a representative combination of an estrogen or estrogen receptor modulator and a compound of formula I

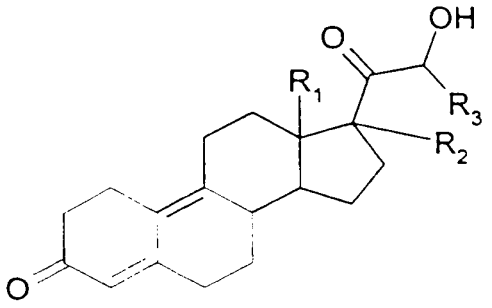


wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, is effective against cerebral degenerative disorders, e.g. Alzheimer's disease, inter alia in mice or rats e.g. Fisher rats or Wistar rats.

These animal models are generally recognized models of cerebral degenerative disorders, e.g. Alzheimer's disease. These data thus indicate that the combination is useful as therapeutic and preventive agents against cerebral degenerative disorders, e.g. Alzheimer's disease, in mammals, including primates such as humans.

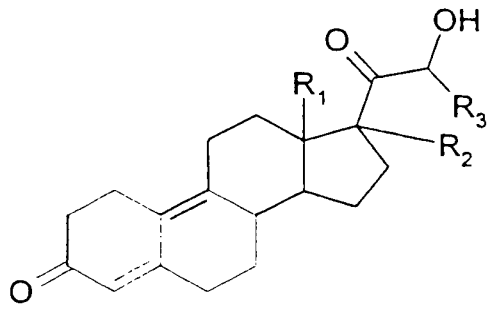
The combination of an estrogen or estrogen receptor modulator and a compound of formula I shows a synergistic effect in treatment of cerebral degenerative disorders and/or a synergistic effect on side-effects, such as cardiovascular disorders, eg. lowering lipids.

In a first aspect the invention relates to a method of treating or preventing cerebral degenerative disorders, preferably Alzheimer's disease, which method comprises administering to a subject an effective amount of an estrogen or estrogen receptor modulator in combination with an effective amount of a compound of formula I



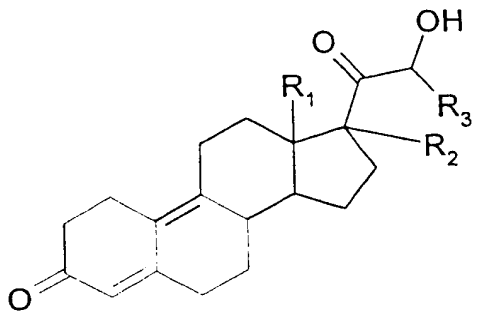
wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, in an amount sufficient to treat or prevent cerebral degenerative disorders.

In a second aspect the invention relates to a kit containing a treatment for cerebral degenerative disorders comprising a) an effective amount of an estrogen or estrogen receptor modulator and a pharmaceutically acceptable carrier in a first unit dosage form; b) an effective amount of a compound of formula I



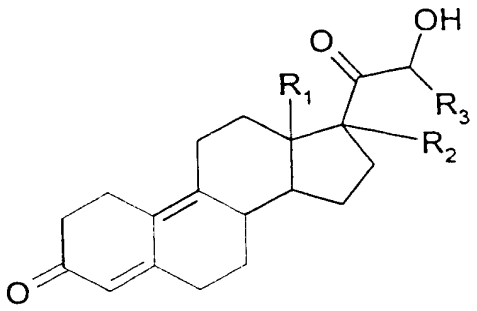
wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier in a second unit dosage form; and c) container means for containing said first and second dosage forms.

In a third aspect the invention relates to a use of an estrogen or estrogen receptor modulator in combination with an effective amount of a compound of formula I



wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for treating or preventing cerebral degenerative disorders.

In a further aspect the invention relates to a composition, such as a pharmaceutical composition, comprising an estrogen or estrogen receptor modulator and a compound of formula I



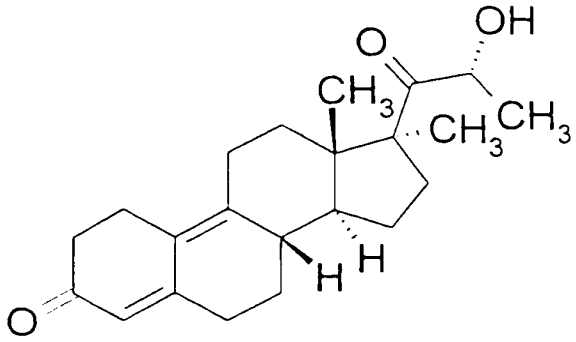
wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

In one embodiment of the present invention the estrogen or estrogen receptor modulator and the compound of formula I is administered simultaneously in one dosage form, preferably orally as a tablet or capsule or as a transdermal patch.

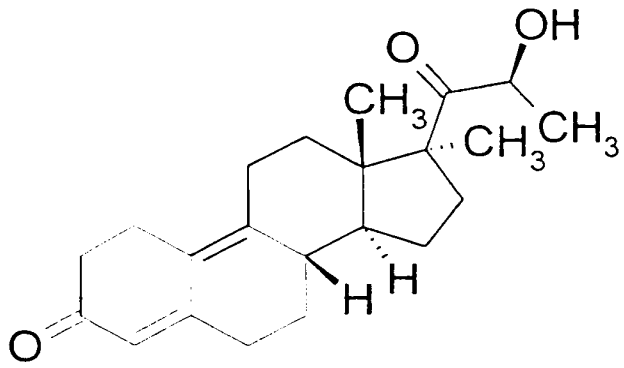
In another embodiment of the present invention the estrogen or estrogen receptor modulator and the compound of formula I is administered substantially simultaneously.

In a further embodiment of the present invention R_1, R_2 and R_3 are independently of each other a C_{1-6}alkyl, such as C_{1-4}alkyl, preferably methyl.

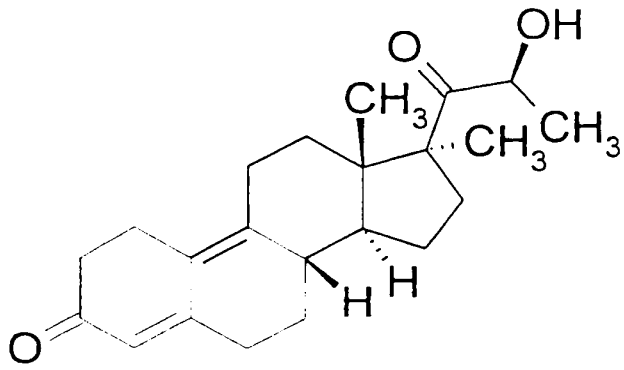
In a further preferred embodiment of the present invention the compound of formula I is selected from



or



In a preferred embodiment of the present invention the compound of formula I is



In a still further embodiment of the present invention the estrogen is selected from 17-beta-estradiol and esters thereof, ethinylestradiol, estriol (trihydroxyestrin), estrone, conjugated estrogens (eg. Premarin), sodium estrone sulfate, 8(9)-dehydroestradiol derivatives, 17alfa-dihydroequilin, equilenin, 17alfa-dihydroequilenin, esterified estrogens, and equilin, prefera-

bly 17-beta-estradiol and esters thereof, ethinylestradiol, and conjugated estrogens. Each of these estrogens is individually considered an embodiment of the invention.

In a further embodiment of the present invention the estrogen receptor modulator is selected from droloxifene, raloxifene, tamoxifen, 4-hydroxy-tamoxifen, idoxifene, centchroman, levormeloxifene, Cis-6-(4-fluoro-phenyl)-5-[4-2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; (-)-Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene -2-ol; Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8- tetrahydronaphthalene -2-ol; Cis-1-[6'-pyrrolidinoethoxy-3'-pyridyl]-2-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene; 1-(4'-Pyrrolidinoethoxyphenyl)-2-(4'-fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline; Cis-6-(4-hydroxyphenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; and 1-(4'-Pyrrolidinoethoxyphenyl)-2-phenyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline. Each of these modulators is individually considered an embodiment of the invention.

In a still further embodiment of the present invention the effective amount of an estrogen or estrogen receptor modulator is from 0.00001 to 1000 mg/day, such as 0.01 to 2.5 mg/day and the effective amount of a compound of formula I is from 0.00001 to 1000 mg/day, such as 0.01 to 1.0 mg/day.

Within the present invention, the estrogen or estrogen receptor modulator and the compound of formula I may be prepared in the form of pharmaceutically acceptable salts, especially acid-addition salts, including salts of organic acids and mineral acids. Examples of such salts include salts of organic acids such as formic acid, fumaric acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, benzoic acid, salicylic acid and the like. Suitable inorganic acid-addition salts include salts of hydrochloric, hydrobromic, sulphuric and phosphoric acids and the like. Each of these salts is individually considered an embodiment of the invention.

The acid addition salts may be obtained as the direct products of compound synthesis. In the alternative, the free base may be dissolved in a suitable solvent containing the appropriate acid, and the salt isolated by evaporating the solvent or otherwise separating the salt and solvent.

The C_{1-12}-alkyl, C_{1-6}-alkyl or C_{1-4}-alkyl groups specified above are intended to include those alkyl or alkylene groups of the designated length in either a linear or branched or cyclic configuration. Examples of linear alkyl are methyl, ethyl, propyl, butyl, pentyl, and hexyl and their corresponding divalent moieties, such as ethylene. Examples of branched alkyl are isopropyl, sec-butyl, tert-butyl, isopentyl, and isohexyl and their corresponding divalent moieties, such as isopropylene. Examples of cyclic alkyl are C_{3-6}-cycloalkyl such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl and their corresponding divalent moieties, such as cyclopropylene.

The compositions and kits of the present invention are useful within human and veterinary medicine, for example, in the treatment or prophylaxis of patients suffering from cerebral degenerative disorders, e.g. Alzheimer's disease. For use within the present invention, the estrogens or estrogen receptor modulators and compounds of formula I and their pharmaceutically acceptable salts are formulated with a pharmaceutically acceptable carrier to provide a medicament for parenteral, oral, nasal, rectal, subdermal or intradermal or transdermal administration according to conventional methods. Formulations may further include one or more diluents, fillers, emulsifiers, preservatives, buffers, excipients, etc. and may be provided in such forms as liquids, powders, emulsions, suppositories, liposomes, transdermal patches, controlled release, dermal implants, tablets, etc. One skilled in this art may formulate the compounds of formula I in an appropriate manner, and in accordance with accepted practices, such as those disclosed in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton, PA, 1990.

Oral administration is preferred. Thus, the estrogen or estrogen receptor modulator and compound of formula I are prepared in a form suitable for oral administration, such as a tablet or capsule, that is either a tablet or capsule containing both the estrogen or estrogen receptor modulator and compound of formula I in one dosage form, or a tablet or capsule containing the estrogen or estrogen receptor modulator in one dosage form and a tablet or capsule containing the compound of formula I in another dosage form. Typically, a pharmaceutically acceptable salt of the compound of formula I is combined with a carrier and moulded into a tablet. Suitable carriers in this regard include starch, sugars, dicalcium phosphate, calcium stearate, magnesium stearate and the like. Such compositions may further include one or more auxiliary substances, such as wetting agents, emulsifiers, preservatives, stabilizers, colouring additives, etc.

Pharmaceutical compositions containing the estrogen or estrogen receptor modulator and the compound of formula I may be administered one or more times per day or week. An effective amount of such a pharmaceutical composition is the amount that provides a clinically significant effect against cerebral degenerative disorders, e.g. Alzheimer's disease. Such amounts will depend, in part, on the particular condition to be treated, age, weight, and general health of the patient, and other factors evident to those skilled in the art.

A typical oral dose will contain a nontoxic dosage range of from about 0.0001 to about 100 mg/kg patient per day of the estrogen or estrogen receptor modulator. A suitable oral dose of a compound of formula I is from 0.0001 to 100 mg/kg patient per day. In sequential regimen oral forms preferred dosages are from 0.001 to 50 mg, in particular 0.01 to 10 mg, more preferred 0.05 to 5 mg, most preferred 0.3 to 2.0 mg estrogen, eg. 17-beta-estradiol or conjugated equine estrogens, continuously combined with 10-14 days of 0.0001 to 10 mg, preferred 0.001 to 10 mg, in particular 0.01 to 5 mg, most preferred 0.05 to 0.5 mg of compound of formula I, eg. a compound of formula II. In continuous regimen oral forms preferred dosages are from 0.001 to 50 mg, in particular 0.01 to 10 mg, more preferred 0.05 to 5 mg, most preferred 0.3 to 2.0 mg estrogen, eg. 17-beta-estradiol or conjugated equine estrogens, continuously combined with 0.0001 to 10 mg, preferred 0.001 to 10 mg, in particular 0.01 to 5 mg, most preferred 0.05 to 0.5 mg of compound of formula I, eg. a compound of formula II, continuously.

A typical transdermal dose will contain a nontoxic dosage range of from about 0.00001 to about 100 mg/kg patient per day of the estrogen or estrogen receptor modulator. A suitable transdermal dose of a compound of formula I is from 0.00001 to 100 mg/kg patient per day. In sequential regimen transdermal forms preferred dosages are from 0.0001 to 50 mg, in particular 0.001 to 1 mg, more preferred 0.01 to 0.5 mg, most preferred 0.02 to 0.1 mg estrogen, eg. 17-beta-estradiol or conjugated equine estrogens, continuously combined with 10-14 days of 0.0001 to 10 mg, preferred 0.001 to 1 mg, in particular 0.01 to 0.5 mg, most preferred 0.05 to 0.4 mg of compound of formula I, eg. a compound of formula II. In continuous regimen transdermal forms preferred dosages are from 0.0001 to 50 mg, in particular 0.001 to 1 mg, more preferred 0.01 to 0.5 mg, most preferred 0.02 to 0.1 mg estrogen, eg. 17-beta-estradiol or conjugated equine estrogens, continuously combined with 0.0001 to 10 mg, preferred 0.001 to 1 mg, in particular 0.01 to 0.5 mg, most preferred 0.05 to 0.4 mg of compound of formula I, eg. a compound of formula II, continuously.

Subject or patient is intended to mean mammals, in particular humans, such as women in the menopause or postmenopausal women.

Treatment as used herein is intended to include profylactic treatment and palliative treatment.

The pharmaceutical compositions containing an estrogen or estrogen receptor modulator and a compound of formula I may be administered in unit dosage form one or more times per day or week. In the alternative, they may be provided as controlled release formulations suitable for dermal implantation. Implants are formulated to provide release of active compound over the desired period of time, which can be up to several years. Controlled-release formulations are disclosed by, for example, Sanders et al., J.Pharm.Sci. 73 (1964), 1294 - 1297, 1984. Controlled-release formulations are also disclosed by U.S. Patent Specification No. 4,489,056; and U.S. Patent Specification No. 4,210,644, which are incorporated herein by reference.

Since the present invention relates to the prevention or treatment of cerebral degenerative disorders, e.g. Alzheimer's disease by treatment with a combination of active ingredients which may be administered separately, the invention also relates to combining separate pharmaceutical compositions in kit form. The kit includes two separate pharmaceutical compositions: in one embodiment an estrogen and a delta4,9-gonadiene-21-ol-3,20-dione of formula I; and in another embodiment an estrogen receptor modulator and a delta4,9-gonadiene-21-ol-3,20-dione of formula I. The kit includes container means for containing the separate compositions such as a divided bottle or a divided foil packet. Typically the kit includes directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g. oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

An example of such a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff material covered with a foil of a preferably transparent plastic material. During the packaging process recesses are formed in the plastic foil. The recesses have the size and shape

of the tablets or capsules to be packed. Next, the tablets or capsules are placed in the recesses and the sheet of relative of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are sealed in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

The term "estrogen" or "estrogens" has its conventional meaning and comprises estrogen and estrogen derivatives such as 17-beta-estradiol and esters thereof, ethinylestradiol, estriol (trihydroxyestrin), estrone, conjugated estrogens e.g. disclosed in US Patent nos 2,720,483 and 2,565,115, in particular premarin cf. internet place www.equinerescue.org/pmu_link.html, sodium estrone sulfate, 8(9)-dehydroestradiol derivatives as disclosed in WO 98/16544, 17alfa-dihydroequilin, equilenin, 17alfa-dihydroequilenin, esterified estrogens, and equilin.

Selective estrogen receptor modulators (SERMs), which previously were characterised as estrogen antagonists/partial agonists on their basis of their binding to the estrogen receptor alpha, act as full estrogen agonists in bone. The acronym SERM takes into account the fact that the activity of these agents is tissue selective and they cannot be definitely labeled as agonsists or antagonists but only as modulators of the estrogen receptor until their actions in specific tissues have been evaluated (Gustafsson, Current Opin Chem Biol 1998; 2:508-511). Thus, the term "SERM"s or "estrogen receptor modulators" has its conventional meaning and comprises droloxifene, raloxifene, tamoxifen, 4-hydroxy-tamoxifen, idoxifene, centchroman, Cis-6-(4-fluoro-phenyl)-5-[4-2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; (-)-Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene -2-ol; Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8- tetrahydronaphthalene -2-ol; Cis-1-[6'-pyrrolidinoethoxy-3'-pyridyl]-2-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene; 1-(4'-Pyrrolidinoethoxyphenyl)-2-(4'-fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline; Cis-6-(4-hydroxyphenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; 1-(4'-Pyrrolidinoethoxyphenyl)-2-phenyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline and the like.

The present invention is further illustrated by the following examples which, however, are not to be construed as limiting the scope of protection. The chemist of ordinary skill in the art will

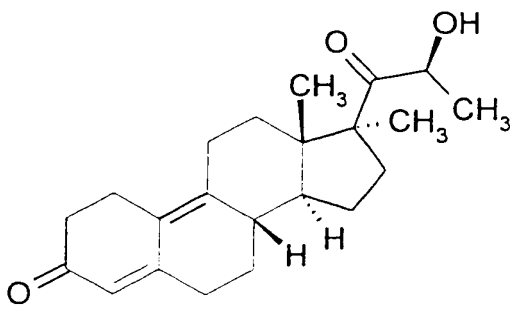
recognize that certain compounds related to this invention will contain one or more atoms which may be in a particular stereochemical or geometric configuration, giving rise to stereoisomers and configurational isomers. All such isomers and mixtures thereof are included within the present invention. Some of the compounds related to the present invention have assymetric carbon atoms and are enantiomers or diastereomers. Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods known per se. Such methods may be chromatografy and/or fractional crystallization. Enantiomers can be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound, eg. alcohol, separating the diastereomers and converting, eg. hydrolyzing, the individual diastereomers to the corresponding pure enantiomers. All such isomers, including diastereomers, enantiomers and mixtures thereof are considered a part of this invention.

The features disclosed in the foregoing description and in the following examples may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

Examples

Conjugated estrogens may be obtained following the process described in US 2,720,483 or US 2,565,115, which are incorporated herein by reference.

The compound having the formula II



(II)

may be prepared as described in US 4,273,771. The compound of formula II is called trimegestone.

An example of a tablet contains a conjugated estrogen (0,6mg) and a compound of formula II (2,4 mg) formulated with pharmaceutically acceptable carriers to provide a medicament for oral administration according to conventional methods. The formulation further include the following diluents, fillers, emulsifiers, preservatives, buffers and/or excipients, that is calcium phosphate tribasic, calcium sulfate, canauba wax, cellulose, glyceryl monooleate, lactose, magnesium stearate, methylcellulose, pharmaceutical glaze, polyethylen glycol, sucrose, povidone, titanium dioxide and red ferric oxide. One skilled in this art may formulate the tablet composition in an appropriate manner, and in accordance with accepted practices, such as those disclosed in Remington's Pharmaceutical Sciences, Gennaro, ed., Mack Publishing Co., Easton, PA, 1990.

TWO-WAY ACTIVE AVOIDANCE (AA) PROTOCOL

Animals and equipment:

Male and female gonadectomized Wistar rats were used (ovaries and testicles were removed at the age of 6 weeks). The animals were allowed to adapt to the laboratory conditions for 1 week prior to the experimental procedure. Food and tap water were freely available in the home cages throughout the studies, but not in the shuttle-boxes. The animals were single sex group-housed with 8 rats per cage. For each session, the animals were transported from the animal quarters to the experimental room. A normal 12 h/12 h light/dark regime was operative (lights on at 06.00 hours) and room temperature was held between 20-23° C. Active avoidance sessions were conducted in standard two-compartment shuttle-boxes (Gemini II Avoidance System; San Diego Instruments Inc.). The separation wall contained a doorway in the middle, measuring 8.5 x 7 cm (w x h). Both compartments (25 x 21 cm) in the shuttle-box had a grid floor that (compartment-wise) could be electrified by a shock-scrambler. Both compartments were equipped with a cue light and a cue beeper. A fan mounted on the back-wall of the shuttle-box provided ventilation and masking noise. For a given subject, all sessions required to complete an AA study took place in the same shuttle-box. After each session, the animals were returned to their respective home cages and transported to the animal quarters.

Experimental procedure:

When the animals were 8 weeks old, a series of 10 acquisition sessions started (1 session per day, on consecutive days, with the exclusion of the weekend). Each session consisted of 40 shock avoidance trials. One hour before each session, different groups of animals (N = 8 per group) were injected SC with estradiol benzoate (50 µg/kg), trimegestone (500 µg/kg), a

combination of estradiol benzoate (50 µg/kg) and trimegestone (500 µg/kg), or with the vehicle only (peanut oil). Animals designated to the same experimental group were run in parallel. After an animal was placed in the shuttle-box, a session started with an adaptation period of 5 min, followed by the simultaneous onset of the cue light and the beeper in the compartment where the animal was located at that time. This signalled to the animal that a shock would follow in that particular compartment. The cue light and beeper provided the animals with a multisensory conditioned stimulus (CS), i.e., a visual stimulus (a white light in an otherwise dark shuttle-box) combined with an auditory stimulus (a tone of approximately 65 dB(A) intensity, when measured in the center of the compartment). The offset of the CS (after 5 sec) initiated the onset of the unconditioned stimulus (UCS), a 220 V- 0.2 mA electrical shock delivered through the grid floor. The UCS was terminated after 2 sec. The CS-UCS sequence was repeated 40 times within a session. The length of the inter-trial intervals was random (in steps of 1 sec), with a mean value of 15 sec, and with the lower- and upper-limit set at 10 and 20 sec, respectively. Session duration was variable and, dependent on the behavior of the animal, between 15 and 20 min.

Measurements:

- Avoidance: The number of crossings (+ latency time) to the other 'safe' compartment within 5 sec after onset of the CS (i.e., before the onset of the UCS). In that case the animals did not receive a shock.
- Escape: The number of crossings (+ latency time) to the other 'safe' compartment within 2 sec after onset of the UCS (i.e., after the offset of the CS, but before the offset of the UCS). In that case the animals did receive a shock, which varied in duration (somewhere in between 0 and 2 sec).
- No Escape: The number of failures to cross to the other 'safe' compartment within 2 sec after onset of the UCS. In that case the animals did receive a shock with a duration of 2 sec.
- Inter-Trial Crossings: The number of crossings between the compartments without the presence of either the CS or UCS. Every inter-trial crossing was punished by a shock delivery.

Analysis:

Acquisition data were submitted to a two-way analysis of variance (ANOVA) with repeated measures, with the between-subjects factor DRUG (4 levels; the 3 different drug conditions plus vehicle treated group), and the within-subjects factor SESSION (10 levels; session 1 through 10). The primary dependent variable was number of avoidance responses. Additional group-wise comparisons were made using ANOVAs with 2 levels of the factor DRUG.

Assessment of pharmacological activity in the Type 2 diabetic mouse model db/db.

Animals:

Male db/db mice were used as a model for type 2 diabetes. The db mouse is of C57BL/KsBom background and has the mutation localized to chromosome 4. The homozygotic db/db mouse is characterized by obesity, hyperphagia, hyperinsulinemia and hyperglycemia. As with most other conditions of hyperinsulinemia, the insulin response to glucose eventually becomes impaired leading ultimately to severe glucose intolerance Due to these phenotypic characteristics this mouse model is recognized as a model of type 2 diabetes. The animals used in these studies were 13 weeks of age and at a time point of hyperinsulinemia and severe hyperglycemia with modest hypertriglyceridemia. All animal procedures were conducted according to Novo Nordisk A/S Animal Care approved protocols, and the experiments were done in compliance with internal animal welfare and national guidelines.

The animals were allowed to adapt to the laboratory conditions for 2 weeks prior to the experimental procedure. Normal chow and tap water were freely available in the home cages throughout the studies. A normal 12 h/12 h light/dark regime was operative (lights on at 06.00 hours) and room temperature was held between 20-23° C.

Experimental procedure:

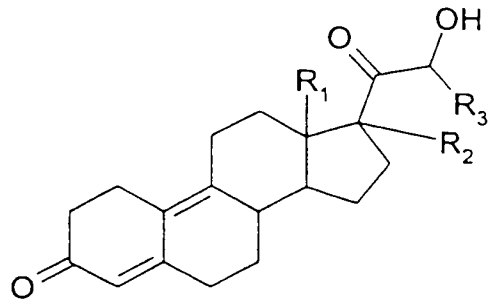
Animals were allocated to respective groups of treatment at the age of 13 weeks and with 6 animals per group. Full-blood glucose (non-fasting) was measured prior to treatment. Different groups of animals were injected SC daily with 17beta-estradiol valerate (0.03 mg/kg), trimegestone (0.30 mg/kg), a combination of 17beta-estradiol valerate (0.03 mg/kg) and trimegestone (0.30 mg/kg), or with the vehicle only (peanut oil). After 7 days of treatment full-blood glucose, serum triglycerides and total serum cholesterol were measured from samples of blood drawn from the retro-orbital sinus in non-fasting animals. An oral glucose tolerance test was performed on day 9 after an overnight fasting. Blood were sampled from the tail vein at time 0 min (baseline) and at 30, 60 and 120 min upon an oral glucose load of 3 g glucose/kg.

Analysis:

Analysis of full-blood glucose, serum triglycerides and total serum cholesterol were performed. Glucose levels in blood samples from the oral glucose tolerance test were used for calculation of the incremental Area Under the Curve ($AUC_{0\text{-}120min\ -\ baseline}$). All data are expressed as percentage change of vehicle treated animals (cf. fig 1).

CLAIMS

1. A method of treating cerebral degenerative disorders which method comprises administering to a subject an effective amount of an estrogen or estrogen receptor modulator in combination with an effective amount of a compound of formula I

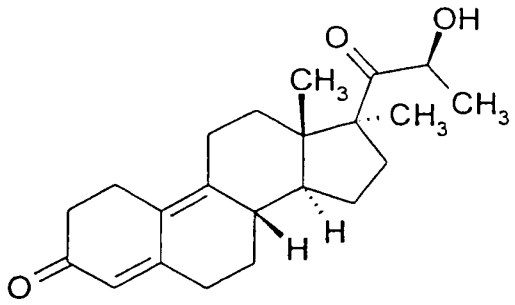


wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, in an amount sufficient to treat or prevent cerebral degenerative disorders.

2. Method according to claim 1 wherein the estrogen or estrogen receptor modulator and the compound of formula I is administered simultaneously in one dosage form, preferably as a tablet or capsule or transdermal patch.

3. Method according to claim 1 wherein the estrogen or estrogen receptor modulator and the compound of formula I is administered substantially simultaneously.

4. Method according to any one of claims 1-3 wherein the compound of formula I is



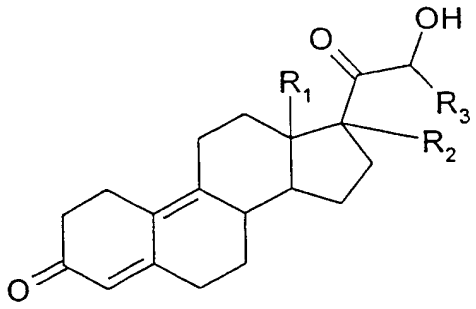
5. Method according to any one of claims 1-4 wherein the estrogen is selected from 17-beta-estradiol and esters thereof, ethinylestradiol, estriol (trihydroxyestrin), estrone, conjugated

estrogens, sodium estrone sulfate, 8(9)-dehydroestradiol derivatives, 17alfa-dihydroequilin, equilenin, 17alfa-dihydroequilenin, esterified estrogens, and equilin.

6. Method according to any one of claims 1-4 wherein the estrogen receptor modulator is selected from droloxifene, raloxifene, tamoxifen, 4-hydroxy-tamoxifen, idoxifene, centchroman, Cis-6-(4-fluoro-phenyl)-5-[4-2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; (-)-Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene -2-ol; Cis-6-phenyl-5-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-5,6,7,8- tetrahydronaphthalene -2-ol; Cis-1-[6'-pyrrolidinoethoxy-3'-pyridyl]-2-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene; 1-(4'-Pyrrolidinoethoxyphenyl)-2-(4'-fluorophenyl)-6-hydroxy-1,2,3,4-tetrahydroisoquinoline; Cis-6-(4-hydroxyphenyl)-5-[4-(2-piperidin-1-yl-ethoxy)-phenyl]-5,6,7,8-tetrahydronaphthalene-2-ol; and 1-(4'-Pyrrolidinoethoxyphenyl)-2-phenyl-6-hydroxy-1,2,3,4-tetrahydroisoquinoline.

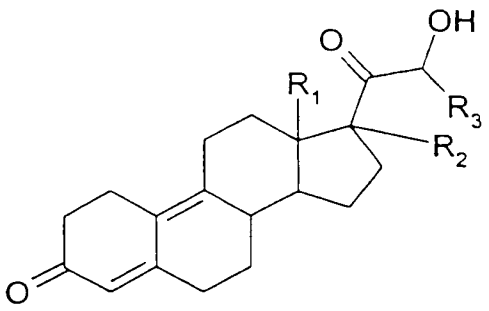
7. Method according to any one of claims 1-6 wherein the effective amount of an estrogen or estrogen receptor modulator is from 0.0001 to 1000 mg/day and the effective amount of a compound of formula I is from 0.0001 to 1000 mg/day.

8. A kit containing a treatment for cerebral degenerative disorders comprising a) an effective amount of an estrogen or estrogen receptor modulator and a pharmaceutically acceptable carrier in a first unit dosage form; b) an effective amount of a compound of formula I



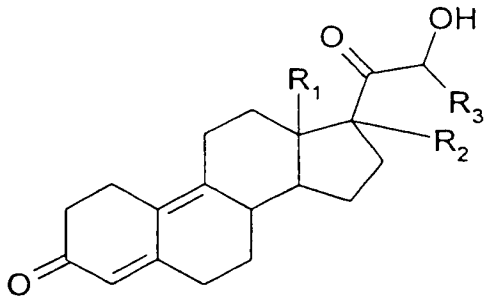
wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier in a second unit dosage form; and c) container means for containing said first and second dosage forms.

9. Use of an estrogen or estrogen receptor modulator in combination with an effective amount of a compound of formula I



wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for treating cerebral degenerative disorders.

10. A composition comprising an estrogen or estrogen receptor modulator and a compound of formula I



wherein R_1, R_2 and R_3 independently of each other are C_{1-12}alkyl, in the form of 21R or 21S epimers or mixtures thereof, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

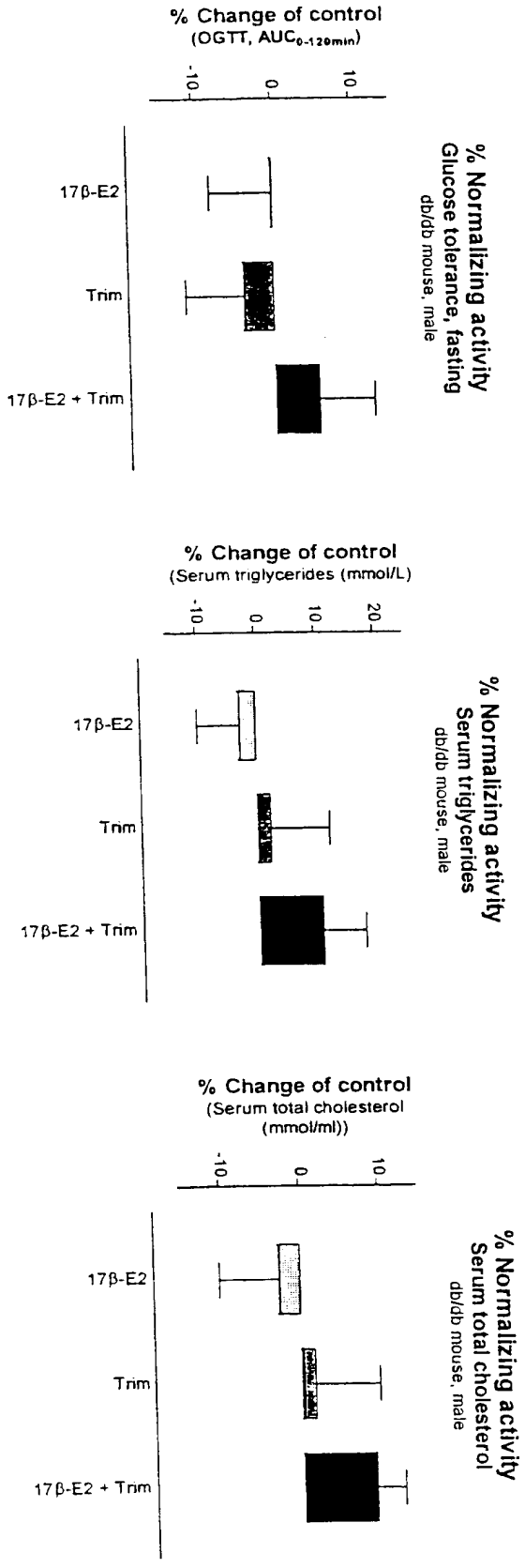

% Change of control
(OGTT, AUC0-120min)
-10
0
10
17β-E2
Trim
17β-E2 + Trim
% Normalizing activity
Glucose tolerance, fasting
db/db mouse, male
% Change of control
(Serum triglycerides (mmol/L)
-10
0
10
20
17β-E2
Trim
17β-E2 + Trim
% Normalizing activity
Serum triglycerides
db/db mouse, male
% Change of control
(Serum total cholesterol
(mmol/ml))
-10
0
10
17β-E2
Trim
17β-E2 + Trim
% Normalizing activity
Serum total cholesterol
db/db mouse, male

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 00/00020

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/565, A61K 31/575, A61P 25/28
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DK 9804246 WO (AMERICAN HOME PRODUCTS CORPORATION), 5 February 1998 (05.02.98) --	10
A	MATURITAS, Volume 31, 1999, A.R. Genazzani, "Menopause and the central nervous system: intervention options" page 103 - page 110 --	1-10
A	Trends in erndocrinology and metabolism, Volume 9, 1998, Nilsson, Stefan et al, "ER BETA: a Novel Estrogen Receptor Offers the Potential for New Drug Development" page 387 - page 395 -- --------	1-10

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 June 2000	16 -06- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/gh Telephone No. + 46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00020

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-7
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK00/00020

Claims 1-7 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/ Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/DK 00/00020

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DK 9804246 WO	05/02/98	NONE	